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RESISTANCE TO *CRONARTIUM RIBICOLA* IN *PINUS MONTICOLA*: STRUCTURE AND GAIN OF RESISTANCE IN THE SECOND GENERATION

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ABSTRACT

Nearly 66 percent of second generation (F_2) seedlings of western white pine, Pinus monticola, *were healthy 2-1/2 years after inoculation with the blister rust fungus,* Cronartium ribicola; *resistance to the pathogen about doubled that of the first generation (F_1). Although three resistance mechanisms were involved, most resistance was due to one, premature shedding of infected needles. Methods for incorporating all resistance mechanisms into a useful product are discussed. The percentage of healthy seedlings from a stand collection of eastern white pine,* Pinus strobus, *included for comparison, was about 5 percent.*

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Research and development toward production of western white pine (*Pinus monticola*) planting stock resistant to blister rust (*Cronartium ribicola*) has been underway since 1950. By 1957, it had become apparent that resistance is under genetic control and is transmissible from rare, rust-free "candidate trees" in rust-decimated stands to their control-pollinated, first-generation (F_1) offspring. Certain candidates were found to embody general combining ability (GCA) for resistance; that is, in a number of crosses, they imparted to their F_1 offspring a consistent above-average level of resistance. Furthermore, when two such "GCA-trees" were mated and the 2-year-old F_1 seedlings from the crosses were artificially inoculated with the rust, resistance of the F_1 was even higher. An average of about 30 percent more of these "GCA-F_1" seedlings withstood artificial exposure to *C. ribicola* than did seedlings from rust-infected trees in rust-decimated stands. Preliminary heritability (h^2) and genetic gain analyses indicated that besides the 30 percent gain from mating GCA-trees, we could expect another 20 percent plus gain from matings of resistance seedlings derived from GCA-trees; i.e., crosses of select F_1 seedlings to produce F_2 (Bingham and others 1960).

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Subsequent progeny tests and more refined and precise h^2 and gain analyses (1960-1970) have given lower estimates of gain for the second generation. First-generation resistance, actually determined in the progeny tests, accrued both from natural selection by the rust and our artificial selection of GCA trees. This resistance remained nearly the same (average 24 percent) when GCA-F_1 seedlings were inoculated at 2 years (Becker and Marsden 1972), but when GCA F_1 seedlings were inoculated at 1 year, it dropped to about 12 percent (Bingham and others 1969). Similarly, estimated second-generation gains from mating resistant GCA-F_1 trees lowered drastically to about 3 percent (Bingham and others 1969), and sometimes to even less than 1 percent (Becker and Marsden 1972). To summarize, under selection for GCA, various progeny tests and h^2 and gain analyses have given three estimates of total gain from the original pre-blister rust population through the second generation of breeding, ca. 14 percent, 27 percent, and 50 percent.

These estimates were sufficiently encouraging for the USDA Forest Service to continue developmental work through selection for GCA among 400 candidate trees and establishment of orchards to mass-produce F_2 seed on resistant GCA-F_1 seedling foundation stocks. Meanwhile, it has been expedient to obtain experimental evidence as to actual gain through the second generation. Toward this end, we have been preserving rust-resistant GCA-F_1 seedlings from progeny tests since 1952. These F_1 trees are accumulated in a resistance-testing arboretum at Moscow, Idaho. Significant levels of female and male fruiting commenced when F_1's were about 10 and 15 years old, respectively. F_2 seed progenies, similar to those we expect from the seed orchards, were obtained by 1966 and sown in the Moscow resistance test nursery that fall.

More recently, we have found a genetic explanation for the resistance phenomenon termed "the spots-only syndrome." Here, foliar infections are not followed by the appearance of bark cankers. We hypothesize that resistance is controlled by two recessive genes acting sequentially in the needles (McDonald and Hoff 1971a,b; Hoff and McDonald 1971).

The purpose of this paper is to report the actual gain realized in artificially-inoculated F_2 seedlings produced under selection for GCA, and to show the kinds and proportions of resistance involved in this gain and ultimately in the F_2 seed orchards.

MATERIALS AND METHODS

Test materials reported upon here include almost 7,500 white pine seedlings represent...ng 49 different control- or wind-pollinated progenies. The bulk of the seedlings are western white pines, but 100 seedlings from one wind-pollinated eastern white pine (*P. strobus*) progeny are included to permit a species comparison.

Susceptible western white pine progenies came from mixtures of seed from five or more heavily infected trees residual on six different, rust-decimated "selection areas" in northern Idaho and northwestern Montana. Six seed collections yielded 546 seedlings.[2] At the time seed was collected, these selection-area stands were nearly 100 percent infected and had already been reduced from 30 to 80 percent by rust-caused mortality. The F_1 progenies came from controlled pollinations of 20 relatively rust-free "candidate trees" in the same or similar selection areas. Ten crosses produced 2,876 seedlings. Both parents of the F_1 progenies had been previously progeny tested and rated as exhibiting general combining ability for resistance, as demonstrated by performance of four or more F_1 tester progenies (Bingham and others 1969). The F_2 progenies came from controlled pollination of 40 resistant F_1 trees in families where both natural-stand parents were rated as GCA trees. Thirty-two crosses produced 3,061 seedlings.

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[2]Two collections of seed were from squirrel caches, four lots were from wind-pollinated groups of five trees infected with blister rust.

Eastern white pine seedlings came from seed collected in one otherwise undescribed Michigan stand.

F_1 seed and seed from three susceptible lots were planted in three randomized complete blocks and grown in four nursery beds at a spacing of 2 by 2 inches. The F_2 crosses, susceptible lots, and the eastern white pine collection were planted in 10 randomized complete blocks in one bed, adjacent to the F_1 crosses; spacing was 1-1/2 by 1-1/2 inches.

The entire test was inoculated during September 1968 in one polyethylene-film chamber covered by canvas (Bingham 1972). The inoculum originated from blister rust-infected, telia-bearing leaves of *Ribes hudsonianum* var. *petiolare* from Hobo Creek, about 10 air miles northeast of Clarkia, Idaho. The leaves were arranged, telia down, in a dense layer covering about four-fifths of the surfaces of hardware cloth screens, placed 18 inches above the seedlings in the nursery beds. The interior of the chambers (including seedbeds and seedlings) was drenched with tapwater. The chambers were sealed to maintain relative humidity at 68 to 100 percent (except for 30 hours at 98 to 100 percent) at ambient temperatures (minimum 51°F maximum 84°F, mostly 60-70°F). The inoculation period was 116 hours.

Dates of inspections and results noted were as follows: June 1969, 9 months after inoculation, needle spots present; September 1969, 1 year after inoculation, needle spots and stem symptoms present; September 1970, 2 years after inoculation, stem symptoms present; March 1971, 2-1/2 years after inoculation, stem symptoms present. Seedlings that did not have spots 9 months after inoculations were not used in the data base; previous observations have indicated that under optimal inoculation levels and inoculation conditions nearly 100 percent of the seedlings have developed needle spots by this time (McDonald and Hoff 1971a).

The mechanisms of resistance that lead to rust exclusion were partitioned in the following manner.

1.--Premature shedding of infected needles. This mechanism is exemplified by seedlings that had needle spots 9 months after inoculation, but lost the infected needles within 12 months after inoculation. Consequently, the fungus was excluded before it reached the stem. It is hypothesized that this trait is controlled by a single recessive gene (McDonald and Hoff 1971a,b).

2.--Fungicidal short shoot reaction. This mechanism is exemplified by seedlings that had needle spots 9 and 12 months after inoculation, but no cankers nor other stem symptoms (such as bark reactions) developed. Apparently, no stem symptoms developed because the fungus was killed in the short shoot or contiguous stem tissues (Hoff and McDonald 1971). It is hypothesized that this trait is controlled by a single recessive gene (McDonald and Hoff 1971b).

3.--Bark reactions or reactions in the stem that apparently cause the death of the fungus. Seedlings in this category had needle spots at 9 and 12 months. Then, at 12, 24, or 30 months after inoculation, they produced (a) typical, discolored bark cankers along with bark reactions or (b) bark reactions alone. Apparently, the reaction had somehow killed the fungus. The genetics of this trait are unknown, but the reactions can be placed into definite categories, an indication that only a few genes may be involved.

RESULTS

Pinus monticola

The percentage of uninfected F_2 seedlings 2-1/2 years after inoculation was about 66 percent (table 1). When compared to the percentage of uninfected F_1 seedlings in the same test, a gain of about 33 percent was realized (table 2). Also, the gain between the selected F_1 parents and the susceptible lots was substantial (nearly 14 percent).

The level of resistance in the susceptible *P. monticola* lots was about 19 percent. However, there is good reason to believe that the greatest part of this resistance comes from intense natural selection in the high-rust-mortality selection areas. Although there is little information as to the amount of resistance in populations of western white pine that have had no mortality attributed to blister rust, available data indicate that only 3 to 5 percent of the progeny from such stands would be free of cankers (Mielke 1943; U.S. Dep. Agric. 1966). If these percentages are appropriate, the realized gain[3] between a remnant population and a population before any rust mortality might account for most of the 14 to 16 percent difference.

Resistance data (Bingham and others 1960; Bingham and others 1969; and Becker and Marsden 1972) were based on the percentage of healthy seedlings; i.e., on seedlings that somehow withstood rust and were not diseased 2 or more years after inoculation. However, we now believe that there are several resistance genes and mechanisms of resistance in western white pine, any one of which can result in healthy seedlings. Consequently, we are interested in determining how the frequency of various genes or mechanisms of resistance is affected when selection for GCA is based on a percentage healthy criterion.

Tables 1 and 2 show that most of the gain in the susceptible lots in the F_1 and F_2 was due to the premature needle-shedding mechanism. The percentage gain of the F_1 selections above the susceptible lots was nearly equal for the three resistance mechanisms (table 2); however, the increase of the premature needle-shedding trait from the F_1 to the F_2 generation appeared to be very large when compared to the other mechanisms of resistance.

Since we have hypothesized that the first two resistance mechanisms are controlled by single recessive genes, we can look at the genetic gain by determining the frequency of the genes. The gene frequency for both the premature shedding and the fungicidal short shoot gene is the square root of the proportion of healthy seedlings of each factor. For the premature shedding gene, the gene frequency is 0.38 for the susceptible lots, 0.45 for the F_1 generation, and 0.69 for the F_2 generation. For the fungicidal short shoot gene, the gene frequency is 0.15 for the susceptible lots, 0.25 for the F_1 generation, and 0.47 for the F_2 generation. Therefore, the genetic gain from the susceptible lots to the F_2 generation is about equal.

Pinus strobus

Unselected *P. strobus* exhibited a low level of resistance (table 1); we observed only two of the resistance traits, premature needle shedding and bark reactions. Also, the amount of mortality was very high (79.8 percent) compared to that of western white pine (average mortality for all crosses was 14.2 percent).

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[3]Following intense natural selection after 80-90 percent rust mortality.

Table 1.--*The level and the mechanisms of resistance of* Pinus monticola *and* P. strobus *to white pine blister rust 2-1/2 years after inoculation*

Species	Type of parentage	Lots or crosses	Total seedlings	Seedlings uninfected	Mechanism of resistance: Premature shedding of infected needles	Mechanism of resistance: Fungicidal short shoot	Mechanism of resistance: Bark reaction	Killed by blister rust	Seedlings with no spots nor stem symptoms (probable escapes)[1]
		No.	*No.*	*Percent*	*Percent*	*Percent*	*Percent*	*Percent*	*No.*
P. monticola	Susceptible	6	546	18.9	14.5	2.0	2.4	17.8	23
	F_1	10	2,876	32.7	19.9	5.2	7.7	13.8	35
	F_2	32	3,061	65.8	47.4	11.5	6.9	7.0	462
P. strobus	P_s	1	99	5.0	3.0	0	2.0	79.8	0

[1]These seedlings were not included in the data base. They are included here just to indicate the level of "disease escape" in this test.

Table 2.--*Resistance and realized gain in resistance to white pine blister rust in various lots and/or crosses in* Pinus monticola

Resistance category	Resistance			Realized gain	
	Susceptible lots	F_1	F_2	From susceptible to F_1	From F_1 to F_2 generation
	Percent				
Total seedlings uninfected	18.9	32.7	65.8	13.8	33.1
MECHANISMS OF RESISTANCE					
Premature shedding	14.5	19.9	47.4	5.4	27.5
Fungicidal short shoot[1]	2.4	6.5	21.9	4.1	15.4
Bark[1] reaction	2.9	10.3	16.8	7.4	6.5

[1]Corrected for the number of resistant seedlings due to the previous mechanism(s) of resistance.

DISCUSSION

Nearly 66 percent of the western white pine seedlings from the F_2 generation that exhibited needle infections were uninfected 2-1/2 years after inoculation. This increase (about 33 percent) over the F_1 generation was a much higher gain in resistance than had been predicted. A ready explanation for this rapid gain is that the two main mechanisms of resistance involved are controlled by single genes. On the other hand, if these single genes are recessive, as we have hypothesized, then an even greater gain should have been realized. In fact, since we hypothesized that certain mated F_1 parents were likely to be homozygous for one or the other of the recessive resistance genes, we should have seen some families that were 100 percent resistant. However, the highest resistance observed was only 88 percent. Even though the F_1 parents were selected 15 years ago--long before the single genes were hypothesized--we found this figure somewhat disappointing; we had hoped the F_2 data would confirm our hypotheses concerning single genes.

We can propose several reasons why certain of the F_2 progenies did not have as high a resistance as we predicted on the basis of the single-gene-controlled traits:

1.--F_1 parents that had the same homozygous recessive genome were not included in the random crosses, which seems unlikely since most of the resistance was due to one gene (premature shedding). Also, in the F_2's from F_1 selfing (only three progenies in the test), the pattern of resistance reflected the F_2 data for progenies from outcrossed F_1's.

2.--One report indicates that the two needle resistance mechanisms can be bypassed by direct penetration of the stem; Van Arsdel (1968) successfully inoculated very tender young shoots of eastern white pine. However, direct penetration of stems has never been demonstrated on *P. monticola* here in Moscow in 20 years of progeny testing.

3.--The inoculated 2-year-old seedlings may have borne some primary foliage that is relatively much more susceptible. *P. monticola* seedlings inoculated at 1 year are highly susceptible compared to seedlings from the same or similar type cross inoculated at 2 years (Bingham 1972). At the time of inoculation, the main morphological difference between the two age groups is the presence of mostly primary needles on the 1-year-old seedlings and mostly secondary needles on the 2-year-old seedlings. However, there is often some primary foliage on normal 2-year-old seedlings. These simple leaves also are produced on seedlings 2 years old or older because of mechanical injury or physiological disturbances on lammas growth.

4.--The genes are not completely recessive. This fact should also explain why susceptible lots contained a relatively high number of seedlings carrying the genes that controlled the premature needle-shedding and fungicidal short shoot traits.

5.--New pathogenic races.

Determination of the complete genetic structure of blister rust resistance in western white pine must await further research. Meanwhile, breeding for resistance need not be delayed. Rapid progress can be made by using the system of selection for GCA proposed by Bingham, Squillace, and Wright (1960). However, this somewhat "blind" selection could result in the situation encountered in the experimental F_2 progenies reported in this paper; i.e., about 70 percent of the apparent resistance is controlled by the single recessive needle-shedding gene. For this reason, we propose that a more conservative selection method be used. More often than not, single gene resistance is overcome by new pathogenic races.

We propose that the selection be based on individual mechanisms of resistance and that an effort be made to incorporate as wide a range of mechanisms as possible into the new resistant populations. In this paper, we have discussed three mechanisms. Two of these appear to be typical vertical resistance factors, expressed as major gene inheritance and exclusion of the rust. The third (bark reactions) also excludes the rust eventually, often after 2 or 3 years, but inheritance seems to be oligogenic or possibly polygenic. In addition, several other resistance mechanisms have been observed in western white pine (Bingham and others 1971; Hoff and McDonald 1972). Some of these are race specific, others are horizontal resistance mechanisms; e.g., fewer needle spots, slow fungus growth. At present, we would advocate incorporating all these forms of resistance, emphasizing horizontal resistance mechanisms as much as possible.

In 1971, seed orchards for rust-resistant western white pine were started near Coeur d'Alene, Idaho. The selection method used was based on individual mechanisms of resistance. An effort was made to select equal numbers of seedlings (from many crosses) showing premature needle shedding, fungicidal short shoot, and bark reactions. When possible, seedlings were also chosen because they exhibited fewer needle spots and were from low-needle-spotting families or were from families that exhibited other horizontal resistance factors. Seed from these orchards should contain a fair amount of genetic variability for resistance at a level useful for artificial regeneration of western white pine.

The eastern white pine seed lot included in the test was found to be highly susceptible. Although we have no information concerning the history of this stand collection, we can assume either that the stand had not been reduced by blister rust

mortality or that resistance did not hold up in a new environment. We favor the first explanation. From time to time, we have tested several other species of white pine that were ranked as being highly resistant; they have retained their resistance in our tests.

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